IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
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Akira IMAIZUMI et al.	)	Group Art Unit: 1652
	)	
Patent Application No.: **10/023711**	)	
	)	Examiner: Christian Fronda
Filed: December 21, 2001	)	
	)	
For: METHOD OF PRODUCING TARGET SUBSTANCE BY FERMENTATION	)	Confirmation No.: 6895
	)	

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Akira Imaizumi, state that I am one of the named applicants of the above-mentioned application and am one of the co-inventors of the subject matter described and claimed therein. With this declaration, data demonstrating the effect of *rmf* gene disruption on L-tryptophan production is submitted for consideration. Such data, combined with that presented in the specification showing effective production of L-lysine and that presented in the previously filed declaration under 37 C.F.R. §1.132 showing effective production of L-glutamic acid by *rmf* gene disruption, clearly provide sufficient evidence to support the genus claim of production of any L-amino acid as recited in Claim 1 of the above-captioned U.S. patent application.

2. *Escherichia coli* WA-04 was used to derive the strain in which the *rmf* gene is disrupted. WA04 is a tryptophan-producing strain derived from *E.coli* No.202 (WO2005/103275) by introducing the *scr* operon and deleting the *iclR* gene. The *rmf* gene disruption was performed as described previously (see example 2 in the specification). The resulting disruptant, WA-04R, and WA-04 were grown in pre-seed medium containing 10 g/l Bacto Tryptone (Difco), 5 g/l yeast extract (Difco), 1 g/l sucrose, and 1 g/l NaCl for 8 hr at 30°C.

3. Then, 0.9ml of culture was transferred into a seed medium containing 10 g/l sucrose, 0.5 g/l $MgSO_4$, 1 g/l KH_2PO_4, 2.5 g/l $(NH_4)2SO_4$, 10 mg/l $FeSO_4 \cdot 7H_2O$, 10 mg/l $MnSO_4 \cdot 5H_2O$, 0.4 g/l soybean hydrolysate (Mameno), 50 mg/l L-methionine, 125 mg/l L-phenylalanine, 125 mg/l L-tyrosine, 5 mg/l Thiamine-HCl, and 36.5 mg/l pyridoxine. Cultivation was performed in 1-liter glass jar fermentors (Kojima Tokushu Glass) using the Biott fermentor system at 30°C for 12 hours. pH was adjusted to 6.5 with KOH and maintained at 6.5 during cultivation with NH_3 gas.

4. Then, 30 ml of culture was transferred into a main medium containing 15 g/l sucrose, 0.3 g/l $MgSO_4$, 0.7 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$, 3.13 g/l NH_4Cl, 10 mg/l $FeSO_4 \cdot 7H_2O$, 7.5 mg/l $MnSO_4 \cdot 5H_2O$, 0.75 g/l soybean hydrolysate (Mameno), 300 mg/l L-methionine, 1 g/l L-phenylalanine, 5 mg/l Thiamine-HCl, and 36.5 mg/l pyridoxine. Cultivation was performed in 1-liter glass jar fermentors (Kojima Tokushu Glass) using the Biott fermentor system at 30°C for 40 hours. pH was adjusted to 6.7 with KOH and maintained at 6.7 during cultivation with NH_3 gas. 700 g/l sucrose was fed continuously to maintain the sugar concentration between 1 and 10 g/l.

5. The relative tryptophan accumulation and productivity is shown in the table below. A significant increase of production in WA-04R was also observed.

Table. Relative tryptophan accumulation and productivity of WA-04R.

	Tryptophan accumulation	Tryptophan productivity
WA-04	1.00	1.00
WA-04R	1.20	1.37

6. As an inventor of this invention, I believe that it is clear that the data presented in the specification, and bolstered by the data presented in this declaration and the previously filed declaration, support the full scope of the claims and clearly demonstrates that the applicants, including myself, were in possession of the invention. Production of three L-amino acids by the *rmf* gene-disrupted strain clearly demonstrates possession of the claimed genus since the three exemplified L-amino acids are very diverse in their structure, i.e. L-glutamic acid, L-lysine, and L-tryptophan. For this reason, production of other L-amino acids by the disclosed strains could be expected and/or determined without undue experimentation by a person of skill in the art, such as myself.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that all statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing therefrom.

By: Akira Imaizumi
Akira Imaizumi

Date: May 9, 2006